

Building capacity to implement conservation breeding programs for frogs in Madagascar: Results from year one of Mitsinjo's amphibian husbandry research and captive breeding facility

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Abstract.—Madagascar is ranked 12th in amphibian species richness by the International Union on the Conservation of Nature (IUCN) and is considered to be one of the highest priority countries for amphibian conservation. Nearly one quarter of the island's amphibian species are threatened with extinction with habitat alteration and over-harvesting for the pet trade contributing most to this dramatic decline. The impending threat of the amphibian chytrid fungus *Batrachochytrium dendrobatidis* (Bd), which has been associated with many of the world's recent amphibian population declines and extinctions, is of great concern. In response to the tremendous threats facing Madagascar's amphibians, a national strategy for amphibian conservation was developed, emphasizing the need for *ex situ* conservation action. This project was officially launched through a collaborative effort between a community-run organization, the IUCN, and the Malagasy government. With significant financial support from multiple international agencies, the result was the construction of a captive breeding facility in Andasibe, east-central Madagascar. We discuss the process for developing and implementing this project which has included facility construction, terrarium building, culturing local feeder insects, and the training of Malagasy technicians. This is the first captive breeding and amphibian conservation project of its kind in Madagascar. Our hope is that it will not only serve as a model for other range country facilities, but become a center for training and education in an area of Madagascar that contains tremendous amphibian diversity and endemism.

Key words. Amphibians, Madagascar, husbandry, capacity building, frogs, breeding facility, live food colonies

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Introduction

With more than 286 described frog species (AmphibiaWeb 2012), Madagascar supports among the highest amphibian species richness of any country in the world. All but one frog species are endemic, while salamanders, and caecilians are unknown from the island. The diversity of frog species is highest in the eastern rainforest belt (Andreone et al. 2005), with the area around the village of Andasibe in east-central Madagascar being particularly speciose, supporting more than 100 frog species within a 30 km radius of town (Dolch 2003).

The amphibian faunae around Andasibe and elsewhere in Madagascar is especially amazing in terms of their ecological, morphological, and reproductive diver-

sity (Andreone et al. 2008). For example, the more than 120 species in the subfamily Mantellinae interestingly do not engage in amplexus, and a number exhibit varying forms of parental care. Members of the genus *Mantella* are toxic and display bright aposematic coloration serving as a familiar example of convergent evolution with the poison frog family Dendrobatidae from Central and South America. Containing some of the smallest frogs in the world, species in the genus *Stumpffia* deposit small numbers of eggs in terrestrial foam nests where non-feeding tadpoles develop directly into frogs. The biodiversity of Madagascar is truly impressive, not only in terms of its well-known lemur and plant species, but also in the behavioral and morphological attributes of its diverse amphibian fauna.

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Unfortunately, nearly one quarter of Madagascar's amphibian species are considered threatened with extinction, and an additional 18.5% of species have not yet had their conservation status determined and are listed as Data Deficient (IUCN 2011). The most significant threat facing the frogs of Madagascar is habitat alteration (Andreone et al. 2005; Glaw and Vences 2007), largely due to agricultural activities, charcoal production, logging, and both artisanal and large-scale industrial mining operations. Additionally, particularly charismatic and colorful frog species, such as those in the genera *Dyscophus*, *Mantella*, and *Scaphiophryne*, are at risk from over-harvesting for the international pet trade (Andreone et al. 2006). Of special concern are the Malagasy frog species confined to high altitudes due to the pressing threat of global warming and upslope elevational displacement (Raxworthy 2008).

The threat of emergent infectious diseases is also of grave concern. The amphibian chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*), which has been associated with drastic population declines and extinctions elsewhere in the world, until recently was thought to be absent from Madagascar (Weldon et al. 2008). However, recent indications of *Bd* in the Makay region still remain unconfirmed (Rabemananjara et al. 2011; Andreone et al. 2012). Lötters et al. (2011) conducted an extinction risk assessment based on a combination of environmental models and an examination of species life history traits, and revealed that many of the frog species in Madagascar are likely to be severely affected by *Bd*. Considering this, it is vital to take appropriate biosecurity precautions, develop awareness campaigns, and enact necessary conservation measures as quickly as possible before *Bd* spreads throughout the country.

Captive breeding can be used as a tool for the conservation of amphibians by establishing captive assurance colonies when threats cannot be addressed in time to prevent extinction, and by developing associated reintroduction and population supplementation programs for species in decline (Griffiths and Pavajeau 2008; Mendelson et al. 2007). In recent years, *ex situ* conservation measures for amphibians have notably been applied in direct response to the threat of *Bd* (Pessier 2008). The Amphibian Ark was formed in 2006 to build capacity in range country and subsequently has assembled many tools for helping implement *ex situ* programs (Zippel et al. 2011). Though these programs have limitations and are temporary solutions, in some cases they are the only option available to prevent imminent extinction (Pavajeau et al. 2008).

There are many urgent threats to the endemic frog species in Madagascar, but as of yet there is little capacity to address them through *ex situ* means. A recent survey by García et al. (2008) of zoological institutions and private breeders around the world found only 27 species of frogs from Madagascar were being kept in captivity, and of

these barely more than half (14 species) had reproduced in the last ten years. Furthermore, these programs are largely informal, operating without proper bio-security and population management practices, which are crucial to the long-term success of projects supplying animals for future reintroduction efforts. This knowledge gap and lack of capacity hinders *ex situ* conservation measures. Additionally, until recently, expertise in amphibian husbandry remained outside of Madagascar and this prohibited the development of in-country captive breeding programs. Developing captive breeding programs within the native range of a species is advantageous for numerous reasons, including significantly reducing biosecurity risks, lowering financial costs when compared to exporting species for breeding programs elsewhere, and instilling pride and confidence in range country stakeholders (Gagliardo et al. 2008).

Methods and implementation

ACSAM

To develop a plan to address the threats facing the amphibians of Madagascar, a conference of more than 100 international and Malagasy experts was held in Antananarivo in September, 2006. Known as "A Conservation Strategy for the Amphibians of Madagascar" (ACSAM), this conference led to the development of the *Sahonagasy Action Plan* (Andreone and Randriamahazo 2008) which is now the national strategy for amphibian conservation in Madagascar, endorsed and supported by the Malagasy government. Within this action plan was a call urging a proactive approach to be taken to develop husbandry expertise for frog species from varied ecological guilds, which had yet to be kept in captivity. This would facilitate rapid *ex situ* conservation action should the need arise.

Following ACSAM, the community-run conservation organization Mitsinjo developed a plan to establish a biosecure facility specifically for the purpose of building capacity to maintain, breed, and conserve local amphibian species. Based in the frog diversity hotspot of Andasibe, Mitsinjo is involved in a varied set of activities including research, rainforest restoration, environmental education, ecotourism, and community health components. The organization is composed of approximately 40 members from the Andasibe population, about a dozen of which are employed fulltime.

Mitsinjo identified three main objectives for the breeding facility:

- 1) Build capacity within Mitsinjo and train technicians to care for and manage captive frog populations. Share knowledge and expertise gained with other organizations and institutions in Madagascar.
- 2) Conduct husbandry research on local frog species from varied ecological guilds to understand their life



Figure 1. The facility was constructed between November 2010 and March 2011 from the foundations of an old abandoned forest station. A) Original abandoned building in January 2009. B) Facility construction November 2010. C) Facility construction December 2010. D) Facility construction January 2011.

histories and captive care requirements, facilitating *ex situ* conservation efforts.

3) Establish captive assurance colonies of threatened frog species from the Andasibe-area and develop associated reintroduction and supplementation programs lest they are needed.

Facility specifications and construction

Fundraising began in 2009 and was received first from Amphibian Ark, the Wildlife Conservation Society, and the Association of Zoos and Aquariums. Facility construction began in November 2010, with the basic infrastructure of the building being completed in March 2011 (Figure 1). The facility was constructed in the Mitsinjo-managed Analamazaotra Forest Station from the foundations of an abandoned building historically used for forestry activities. The location was chosen for its elevated position to prevent flooding during the cyclone season and for the ease of access to the main road leading to Andasibe village.

Measuring 185 m², the facility contains three separate areas for live food production, captive breeding and husbandry research, and an isolated room for quarantine

(Figure 2). Entrance to the facility is through two sets of doors, in between which is a threshold on the floor to help prevent organic debris from entering. Beyond the barrier is a hand washing station and area to change into dedicated clothing and footwear. The building was designed to facilitate workflow habits that minimize biosecurity risks, with staff from Amphibian Ark, Woodland Park Zoo, North-West University, and Jersey Zoo contributing input during construction based on experience gained designing similar facilities elsewhere in the world.

Frog species kept at the facility are and will be composed of a local species assemblage, considerably lowering biosecurity risks (Pessier and Mendelson 2010). Water is sourced from a river at Ambatomandondona, which is 2.5 km from the facility. This source is supplemented with rainwater. A solar water heater, 1μ sediment filter, and carbon filtration will be used to help prevent amphibian pathogens from entering the facility through the water supply. Additionally, all windows, doors, and drains are sealed to prevent pests and amphibians from entering or exiting the building. Wastewater is discharged through a carbon and sediment filter to stop soaps, detergents, and chemicals used for cleaning and disinfecting materials from polluting the surrounding forest.

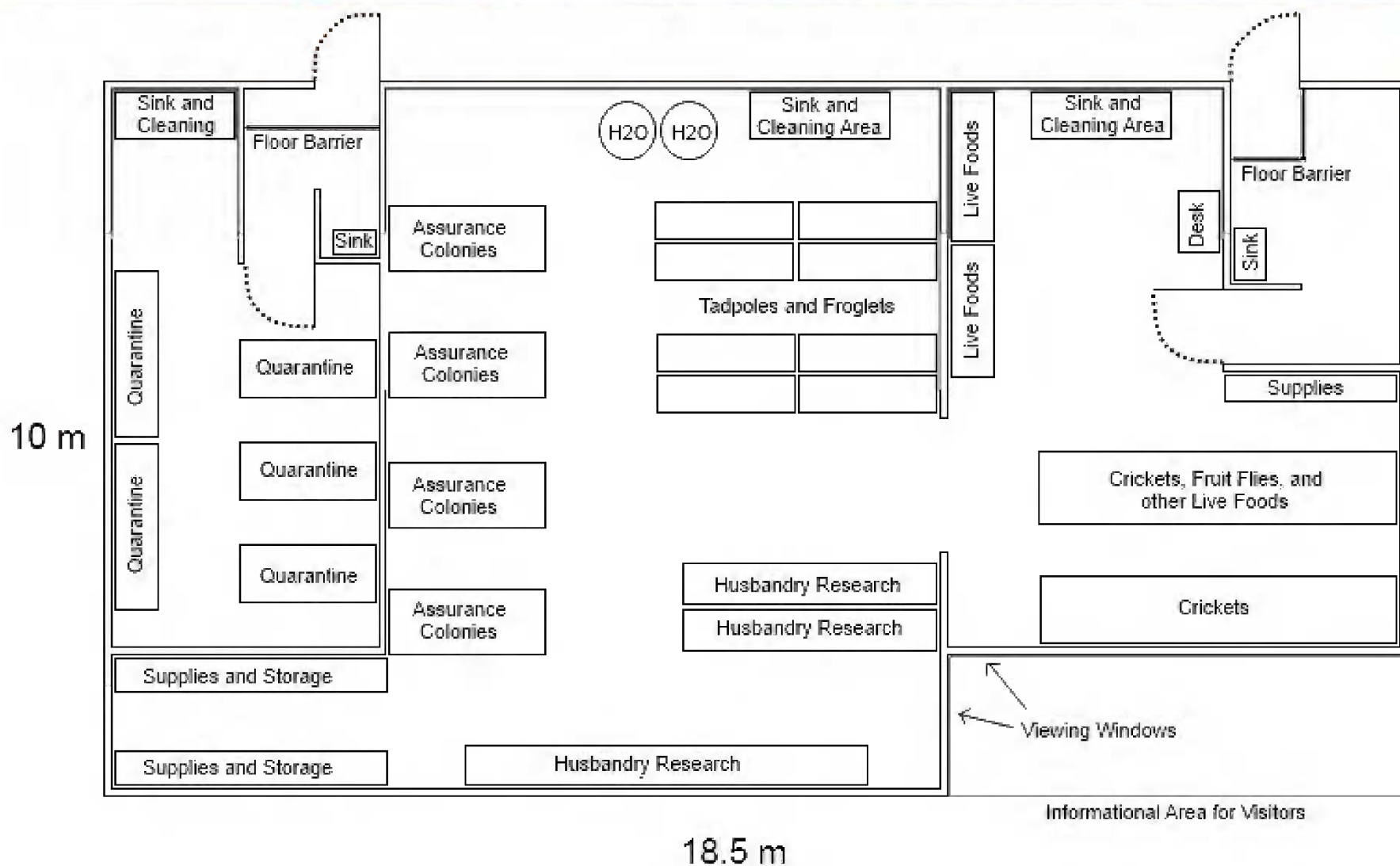


Figure 2. Overview of the biosecure Mitsinjo amphibian captive breeding and husbandry research center as of April 2012.

Materials to build shelves and terraria (wood, glass, silicone, aluminum, screen, etc.) were all sourced from within Madagascar, and were constructed locally in Andasibe. Material used inside terraria such as gravel, dead leaves, and live plants were collected from the surrounding forest when possible. Plants were disinfected with a 0.5% sodium hypochlorite solution before entering the facility, with other organic material being cleaned with water and then fully air dried in the sun for several days prior to being brought inside.

Twenty-four terraria are currently used for rearing tadpoles and offspring with an additional 46 terraria constructed and being used for adult frogs (Figure 3). Terraria are setup in an “open-system” where they are outfitted with bulkheads that drain into floor drains. This allows terraria to be cleaned and serviced without needing to be moved off of shelving units, and helps regulate the moisture content of the substrate. Wastewater from terraria housing captive assurance populations and from terraria for husbandry research drain into separate floor

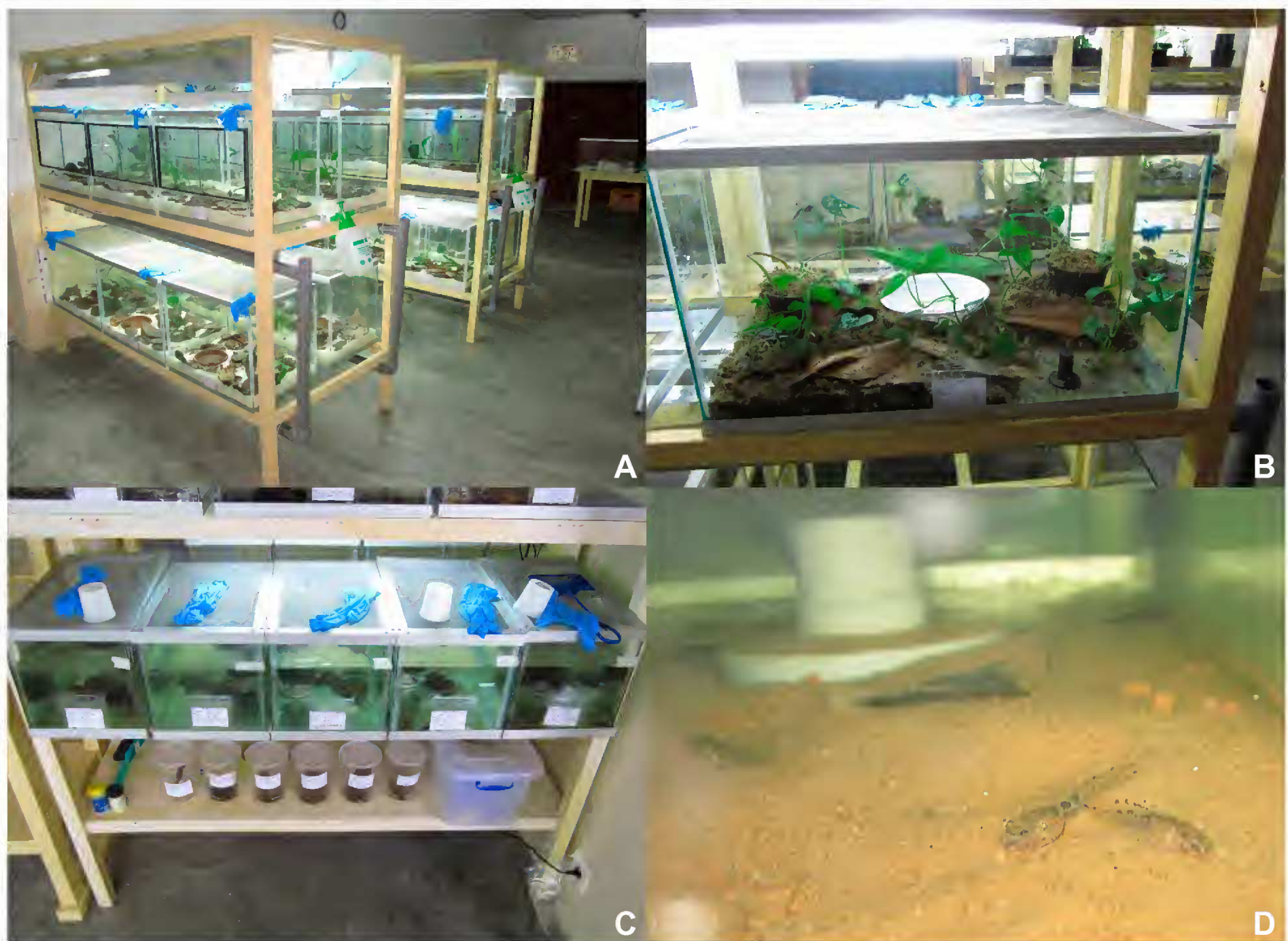


Figure 3. Terraria and aquaria at the breeding facility. A) Terraria setup on shelving and plumbed so wastewater flows into a drain in the floor. B) A terrarium housing a group of *Boophis pyrrhus*. C) Aquaria for raising tadpoles. D) *Boophis pyrrhus* tadpoles produced at the facility.

drains. The facility has capacity and is planned to support a total of 300+ terraria and aquaria, which are continually being built by Mitsinjo and should be finished in 2013.

Mitsinjo's project was officially launched through a Contract of Collaboration with the IUCN SSC Amphibian Specialist Group (ASG) of Madagascar and the Malagasy governmental agency Direction Générale des Forêts (DGF) in April 2011. This contract ensures all activities comply with Malagasy Law and helps make certain Mitsinjo's objectives complement and correspond to those in the *Sahonagasy Action Plan*.

Frog and live food sources

All live foods produced at the facility were originally collected from around Andasibe to prevent introducing potentially invasive invertebrate species to the area. Live food species identification was provided by the University of Antananarivo Department Of Entomology. While the facility was being constructed, more than six months were spent collecting local invertebrates and developing techniques to culture them in captivity. Mitsinjo continues to expand live food sources to provide variation in the

diet of the captive frog populations. Early on, advisors to the project stressed the importance of establishing live food colonies before frogs were brought into captivity. Four frog species were collected and acclimated to captivity in April 2011 once live food cultures were established and the Contract of Collaboration between Mitsinjo, ASG, and the DGF was finalized. The first frogs were assigned to six groups in separate terraria (Table 1). Species were chosen not only for their husbandry research potential, but also to provide Mitsinjo technicians with varied practical experiences caring for taxa with diverse

Table 1. Initial breeding groups established for training in April, 2011.

Group	Species	Males to Females	Breeding?
BLBL-A	<i>Blommersia blommersae</i>	5.0	No
BLBL-B	<i>Blommersia bommersae</i>	5.0	No
BOPY-A	<i>Boophis pyrrhus</i>	3.1	Yes
HEBE-A	<i>Heterixalus betsileo</i>	2.1	No
MABE-A	<i>Mantidactylus betsileanus</i>	3.2	Yes
MABE-B	<i>Mantidactylus betsileanus</i>	4.2	Yes



Figure 4. Seven species of frogs were included in a husbandry research and technician training program during the first year of the project. The IUCN Red List status, in parenthesis, follows species. A) *Heterixalus betsileo* (LC). B) *Mantidactylus betsileanus*, (LC). C) *Heterixalus punctatus* (LC). D) *Blommersia blommersae* (LC). E) *Guibemantis* aff. *albolineatus* “Andasibe” (DD). F) *Stumpffia* sp. “Ranomafana” (DD). G) *Boophis pyrrhus* (LC).

life histories and, presumably, different captive care requirements. Additional individuals of the first four species as well as three new species were enrolled in the program throughout the following year, totaling seven species being kept for training and research as of June, 2012 (Figure 4).

All frogs were collected from or near the road leading to Andasibe village. Two days were spent searching for and collecting target species, after which all frogs were moved into the quarantine room for housing while the final aspects of construction in the main frog room were completed. Body score condition of each individual was recorded weekly during acclimation.

The second group of frogs acclimated to captivity in 2012 was weighed upon entry into and exit out of quarantine. Only after all appeared in good condition, and

there were no unexplained mortalities, were the frogs from the second group moved to the same room, where established populations were being maintained. Detailed records to track their individual identities and sex, health in captivity, collection location, and breeding history are managed in a studbook by Mitsinjo, ASG-Madagascar, and the DGF.

Species currently kept for husbandry research at the facility have either an IUCN Red List status of Least Concern (LC) or Data Deficient (DD), and are not considered priority species for rescue operations by Amphibian Ark. The decision to work with locally abundant LC or DD species was made to manage risks while technicians gained the specialized knowledge and practical experience needed to maintain captive frog populations in a biosecure conservation breeding facility. Information



Figure 5. Lectures and discussions during January-March 2011 helped train Mitsinjo technicians in captive frog husbandry techniques.

and experience gained from maintaining these non-priority species may be applied to establishing captive assurance colonies and developing population supplementation or reintroduction programs for highly threatened species in the future.

Results and discussion

Mitsinjo technician training

To assemble a team of Mitsinjo technicians dedicated to the daily husbandry of amphibians and live food colonies at the facility, a week-long training course was developed in January 2011, which included presentations about basic amphibian biology, ecology, and captive husbandry techniques.

From a group of 14 Mitsinjo members who participated in this initial training course, five technicians were selected to work at the facility and were enrolled in a further two months of intensive preparation with the project's director. Training was composed of assigned readings and related activities about amphibian husbandry, as well as practical lessons involving caring for newly established live food colonies, building terraria, and identifying and handling frog species in the field. As a final component of the training program, a week of on-site presentations and demonstrations about frog husbandry was presented by staff from the Woodland Park Zoo and Amphibian Ark (Figures 5 and 6).

One of the objectives of the project is to build capacity within other Malagasy institutions and organizations to help develop additional amphibian conservation breeding programs elsewhere in Madagascar. As a first step in this direction, a live food production training course supported by Durrell Wildlife Conservation Trust was carried out by Mitsinjo in November 2011 for the University of Antananarivo's Department of Animal Biology. During this week-long course, Mitsinjo technicians instructed a group from the university in techniques de-



Figure 6. A practical hands-on lesson in terraria design and construction, early March, 2011.

veloped to culture local invertebrate species. The newly trained university technicians returned to Antananarivo with starter cultures of live foods to practice culturing them in their laboratory, thereby developing the first set of skills needed to maintain captive frog populations.

Live food production

Fruit flies

Fruit flies (*Drosophila* spp.) were the first live foods established by Mitsinjo, with the earliest successful cultures produced in October, 2010. Two species of different sizes were initially captured, however, only the smaller species (similar in size to the familiar *Drosophila melanogaster*) proved easily cultured. Plastic water bottles covered with fabric secured in place with rubber bands are used to contain the flies (Figure 7), with media being composed entirely of ingredients available locally in Andasibe (Table 2).

Table 2. Fruit fly media (makes 10 cultures)

Ingredient	Quantity
Potatoes-boiled until soft	12-15
Bananas	2
Powdered milk	6 tablespoons
Sugar	2 tablespoons
Baker's yeast	~20-40 granules per culture

Crickets

Trial cricket breeding began in November 2010. Five different species including *Grylloides sigillatus*, one *Gryllus* sp., two *Modicogryllus* sp., and a cave cricket of the family Rhaphidophoridae have been bred by Mitsinjo (Figure 8), but only three are currently producing in quantities large enough to feed captive frogs. Crickets are maintained in ventilated plastic boxes labeled with the hatch date and the species. Boxes measure 60L × 40W ×

30H cm for adult breeders and 35L × 25W × 20H cm for juveniles. The boxes are stored on shelves heated with heat cable which is attached to a thermostat. The temperature varies with season, but typically is maintained between 22 °C and 27 °C. Breeding slowed considerably in 2011 during the cool months of July and August, during which time the facility did not yet have electricity for heating, and nighttime temperatures dropped to as low as 13 °C. Crickets are fed a varied diet of seasonally-available fruits and vegetables (carrot, zucchini, apple, potato, mango, cucumber, etc.) as well as a protein source of ground *patsamena* (a small dried shrimp widely available at markets in Andasibe).

Springtails

The first springtails (*Collembola* sp.) cultured at the facility were sourced from bark on a mango tree in Andasibe village in April, 2011. Attempts were made to culture them on multiple substrates including dead leaves, a soil mixture, and charcoal. Moist charcoal proved to be the most practical. To determine the best food source for the springtails, cultures were divided into two different groups, one fed ground *patsamena* and the other fed Aquafin Professional Basic Fish Flake. Cultures fed fish flake were substantially more productive.

Other live food sources

In addition to fruit flies, crickets, and springtails, Mitsinjo has attempted to establish cultures of various other invertebrates from the Andasibe-area. The most success has been with a local cockroach species from the forest which cannot fly or climb smooth surfaces. They are cared for in nearly an identical way to crickets but are fed a slightly different diet which includes powdered milk. Up to now, only four individuals have been found and collected, and from these founders breeding has only occurred twice, first in October 2011 and then again in January 2012. Currently, Mitsinjo is maintaining a colony of around 60 roaches, most of which are still juveniles. It is expected to take at least one additional year before they are producing enough to be used as a food source for captive frogs.

There has been some success in culturing isopods. These were setup in small plastic boxes layered with moist cardboard and leaf litter, and were fed fish flake. The isopods survived and even appeared to reproduce, but for unknown reasons, all cultures died between June and September 2011. In the future, Mitsinjo plans to again collect isopods and start new cultures.

A small beetle species was also cultured for food. These were originally sourced in grains purchased at



Figure 7. A) Fruit fly cultures on shelves at the facility. B) Fruit flies are cultured in discarded plastic water bottles collected in Andasibe. Fabric is secured in place, over the top with rubber bands, and strips of plastic bag are placed inside (above the media) on which the flies can deposit eggs.

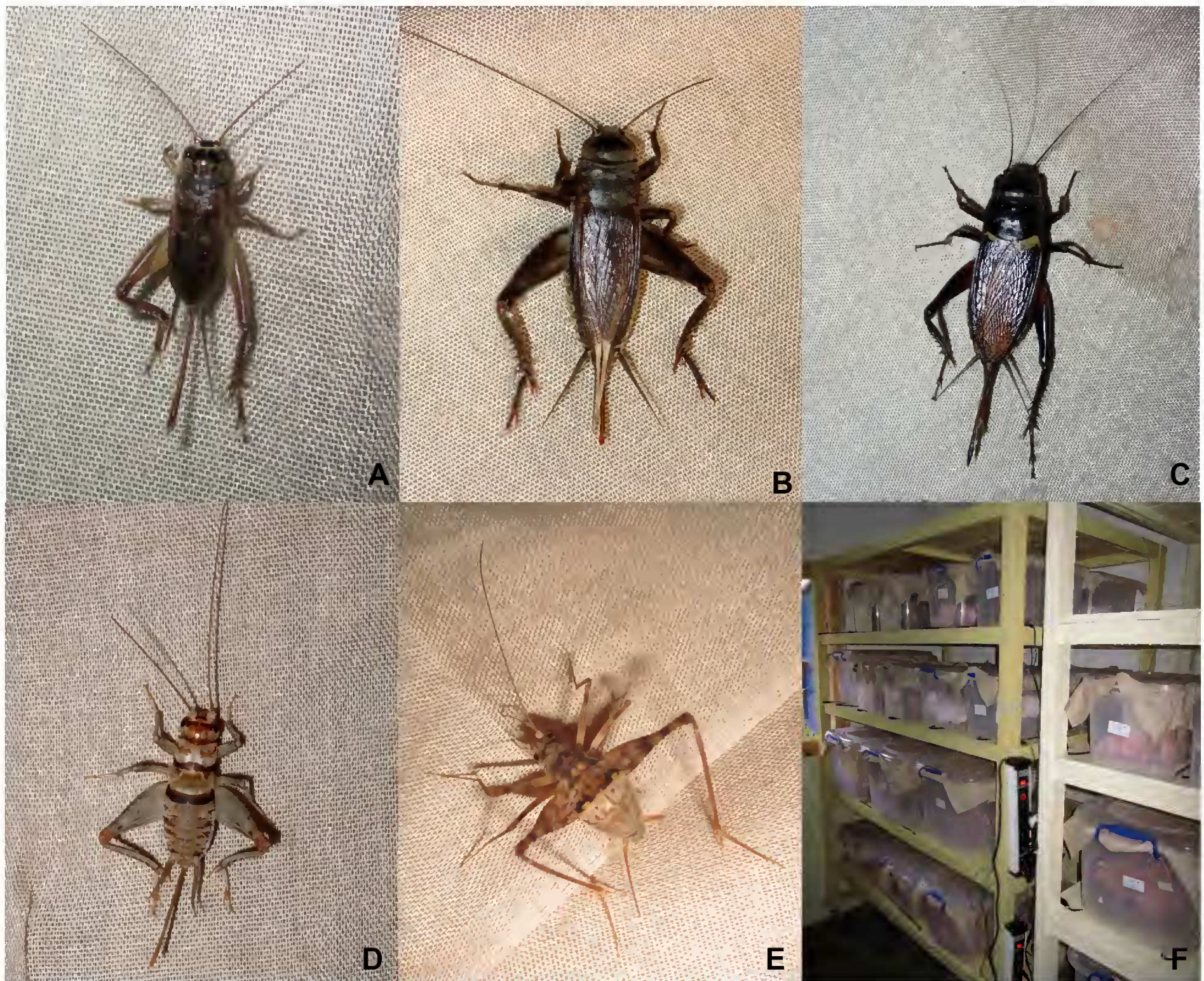


Figure 8. Locally-sourced crickets from Andasibe being bred at Mitsinjo's facility. A) Field cricket (*Modicogryllus* sp.). B) Large field cricket (*Modicogryllus* sp.). C) Large black cricket (*Gryllus* sp.). D) Tropical house cricket (*Gryllodes sigillatus*). E) Cave cricket (*Rhaphidophoridae*). F) Shelves with boxes housing field crickets and tropical house crickets.

market in the village, anticipating that their larvae could be used to vary the diet of small frog species. Unfortunately, they proved to reproduce very slowly, regardless of the media they were kept on (rice, pasta, flour, and peanuts were tried). Additionally, it was time consuming to harvest the larvae from the cultures. As a result, culturing this species was abandoned after one year.

In addition to isopods, cockroaches, and a small beetle species, Mitsinjo attempted to establish an earthworm culture in December 2010. More than 50 worms (species unknown) were collected from soil in Andasibe. Worms were placed into a box containing a mixture of soil and leaf litter. The box was kept outside in a cool location, and the moisture content of the substrate monitored regularly. Vegetable scraps were provided weekly as a food source. While most worms survived, no reproduction was noticed after more than four months and so the culture was discarded. It has recently been brought to our attention that vermiculture operations exist in Madagascar, and it is planned in the coming year to investigate the potential of culturing earthworms as a food source once

again, starting with worms sourced from and using techniques developed by existing vermiculture operations in the area.

Frog husbandry research

The initial four species collected for training and husbandry research remained in good health throughout the first year, with two species (*Boophis pyrrhus* and *Mantidactylus betsileanus*) reproducing on multiple occasions. With no previously published accounts, this may represent the first captive breeding of these frog species. Detailed records of the conditions provided for these species will be disseminated in the future once the captive populations have been maintained for an extended period of time, and hypothesis-driven research has yielded significant results regarding their captive husbandry requirements.

As a first step towards conducting husbandry research on these species, tadpoles from the first clutch of eggs received from *M. betsileanus* were used in a preliminary

training exercise to both help understand the optimal captive larval diet for this species and to train technicians how to conduct hypothesis-driven husbandry studies. Tadpoles were divided into three different aquaria, each one being fed a different diet, with observations made about the metamorphosed frogs which resulted from each group (Figure 9).

Although results from this first pilot-study were statistically inconclusive due to inconsistent data collection and lack of materials to measure and weigh the metamorphosed frogs, it was a beneficial exercise because it allowed technicians to learn how to formulate a hypothesis, collect data, and conduct their own research project. Mitsinjo plans to repeat this same study when *M. betsileanus* breed again, measuring all newly metamorphosed frogs with a caliper and recording all data regarding their development, including when each individual completes metamorphosis.

Conclusions and future outlook

Numerous authors and conservationists have discussed the pressing need to build capacity in Madagascar to manage captive populations of amphibians (Andreone 2006; Furrer 2008; Mendelson and Moore 2008). The development and implementation of the Mitsinjo breeding facility, which is the first project of its kind in Madagascar, is a step in the right direction. However, when considering the large number of individual captive frogs

required to sustain an assurance population of even just one species for 10 years (as described by Schad 2007), and taking this into account alongside the exceptionally high frog species richness found in the Andasibe-area, it would be an enormous task to develop conservation breeding programs for more than a small fraction of the local frog species.

This fact highlights two important points. 1) It is imperative to develop additional capacity in Madagascar with other in-country organizations to manage captive assurance populations of amphibians, as well as to assess the specific conservation needs of species to prioritize those for breeding programs. 2) Captive breeding programs must have exit strategies and complement conservation activities which directly address the most pressing threats facing Madagascar's frogs, such as habitat protection, forest restoration, and environmental awareness and education campaigns.

The outlook for addressing these two points is promising. Notably an Amphibian Husbandry Workshop led by Durrell Wildlife Conservation Trust is scheduled to take place in Antananarivo during December 2012 to train additional organizations and institutions in Madagascar on frog husbandry techniques. This will help build further capacity within Malagasy organizations to manage captive populations of amphibians. Additionally, Mitsinjo is pursuing funding to develop an education and outreach center, which will display live frogs and associated informative graphics to help promote interest in and aware-



Figure 9. Pilot study and training exercise on the optimal larval diet for *Mantidactylus betsileanus*.

ness of the environment. This center will complement Mitsinjo's ongoing environmental education work in Andasibe.

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